



Genomic Approaches for Biodiversity Conservation – A Review

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ABSTRACT

The integration of genomics with biodiversity is promising and reveal valuable information about genome organization and genome evolution. Next Generation Sequencing (NGS) technology generated vast genomic resources like short sequence repeats (SSRs), Single Nucleotide polymorphisms (SNPs), Insertions and deletions (InDels), Copy Number Variations (CNVs), Diversity Array Technology (DART) markers etc. The use of these genomic resources can accelerate biodiversity assessment, conservation, and restoration efforts by enabling scientists to make well-informed decisions. Bioinformatic analyses will further help finding genes, gene products and their function. The genomic technology helps estimating genetic diversity, identification of adaptive alleles, complex diseases and traits, and also enable species identification by DNA barcoding approaches. The use of genomics with classical genetic rescue programs will enable conservation biologists to protect, preserve and manage ecosystems efficiently.

Key Words: Biodiversity conservation, Genome sequence, Genomic resources, Adaptive alleles, Comparative mapping, DNA barcoding

INTRODUCTION

Biodiversity refers to variation at all stages of life and it uncovers species richness.¹³ Biodiversity plays important role in preserving inherent abilities of organisms and the health of an ecosystem. Diversity in life is necessary to face challenges posed by climate changes and diseases, furthermore, it is possible only when we attain a balanced species existence in an ecosystem, our food, shelter are achieved. For example, as we have witnessed a disease outbreak (COVID-19 – Corona Virus Disease - 2019) in the recent past. Therefore, the conservation of biodiversity is of greatest concern as described by Convention on Biological Diversity (CBD). In this review, we focussed on challenges to biodiversity and, the availability of various genomic technologies useful for protecting the biodiversity. The progress in sequencing technology made genomic libraries and genomic DNA clones available that can be used for whole genome sequencing applications. These genomic clones were used for characterizing genomes and to determine the functional aspects of the genomes and its interaction with the environment. As a result, the conservationists were fortified with genomic tools to develop strategies for biodiversity conservation

under varied environmental conditions. Furthermore, the genomic technologies not only help developing various genomic resources but also characterize organisms at molecular level and understand their traits, adaptations to the environment, habitat preferences etc. So far, the genetic or molecular studies have achieved a great extent in unravelling genome diversity and, a plethora of examples of various genomic approaches available and how this information can be transformed to prevent biodiversity loss per se species diversity were thoroughly discussed in this chapter.

Threats to biodiversity:

Environmental threats: The climate change due to water and air pollution, human colonization, expanding urban regions and also invasive species are the leading causes of habitat destruction and biodiversity loss.³¹ It is also evident from a study, the Earth is on its way to sixth mass extinction event due to extensive species loss.⁵ For example, as mentioned in a recent review, over the past 100 years, we have witnessed a 60% increase in plant extinction^{10, 19}. The reasons for the decline of populations are, rapid growth of human societies and development— in particular, land reclamation of coastal regions and aquaculture practices have a negative impact on

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the environment.¹¹ Apart from threats to animal life, major threats to plant life include rapidly expanding industries, new habitat establishments, and over-dependence on timber, excessive use of chemical-based fertilizers in agriculture etc.

Genetic factors: Inbreeding depression (i.e., reduced fitness), deleterious recessive alleles, admixture are genetic factors which have a negative impact on species.¹⁵ Genetic erosion on the other hand also leads to loss of alleles or genes and it occurs due to habitat loss. Other breeding bottlenecks also limit fitness of a species due to genetic incompatibility. In the case of plant species, reduced pollination has impact on gene exchange and lead to reduced fertilization and seed set. Genetic drift, introgression, selection in small or isolated populations and population genetic bottlenecks are few other genetic factors that affect extant of biodiversity.

Thus, the efforts to minimize the rate of extinction and biodiversity loss by In situ and Ex-situ conservation efforts and habitat protection are best practices to prevent this biodiversity loss.²⁹ Policy decisions taken during international treaties such as the Kyoto Protocol and Paris Agreement were also in force to minimize carbon emissions at the same time to mitigate negative impact on various life forms. However, at the species level, it is vital to characterize organisms at molecular level which are capable of tolerating the environmental threats with their inherent and genetic potential by using genomic technologies for understanding their adaptive potential in different environments. In this direction, the application of genome sequencing and molecular technologies play pivotal role in generating molecular data of various organisms. The molecular data arising out of these sequencing efforts help in understanding inherent capabilities of species and help in devising conservation strategies to protect them from losing biodiversity existing within the species.

Sequencing technology: A tool for biodiversity conservation: Sequencing technology started growing from late nineties and sequencing cost drastically reduced while achieving throughput. These developments led to initiation of genome project on a large scale in plants^{7, 8, 16, 20} in the case of animals¹⁴ and, also for bacterial species.²⁷

Initially, it was ABI-Solid and 454 to develop sequencing pipelines, but subsequently, overtaken by Illumina, Pac-bio and, in the recent past, Nanopore technology and single-molecule sequencing technologies have become major ones. Nevertheless, the rapid developments in NGS technology (Table 1) necessitated the upgrading computational power to handle enormous sequence data. However, with the help of bioinformatic tool, numerous reference genomes were developed and millions of genetic variants like SSRs, SNPs, InDels and CNVs were also identified in the genome. The genetic variants are larger in number and are invaluable for taxonomic studies.

The application of reference genomes for conservation studies was attempted.^{4, 15} The reference genome serves as a valuable tool for conservation efforts and serve as a valuable source for design of effective population-level sampling methods (SNP panel, for instance) and are also useful for evolutionary genomic studies as well. RNA sequencing, which is also an outcome of sequencing technology has been an invaluable tool for gene expression studies as RNA experiments and provides a clue about gene expression pattern in different individuals of a species. Various bioinformatic tools that can be used in analyzing the genome are provided (Table 2).

Genomic methods for biodiversity conservation: The world's most collection of plants, animals and microbes are valuable resources for biodiversity conservation and they are a rich source of genes controlling valuable traits. Therefore, any scientific and technological effort that prevents loss of these genes from populations leads to biodiversity conservation to a large great extent. Although, the genetic resources are always rescued by traditional practices like collection and conservation, biotechnological approaches seems to be redefining these traditional approaches. For instance, genomic libraries are an alternative to seed banks for conserving plant genetic resources. Similarly, in the case of animals, sperms or eggs are preserved in freezers generally at low temperatures (-196°C) as part of conservation. The "Cryo banks" is one such effort to store embryos of animals in liquid nitrogen at -196°C for future use and also for the species which are facing extinction. In the case of plant/ crop species, plant tissue cultures are valuable tissue banks consisting of meristematic cells and species that are not propagated by seeds. A few other conservation efforts include cryopreservation and micropropagation.

The first step in studying and preserving biodiversity is the identification of species present in an ecosystem and understands its evolutionary history. The conventional morphological approaches can be limited to identify species in an ecological niche, as efforts to collect specimen for species identification is carried out. This may be due to some limitations at organism level or due to rare sight of that species in the environment. In these cases, biotechnological approaches offer possibility as a meagre sample availability of a particular species such as animal excretions, shedding from the body parts etc. can become a valuable sample. One such technological advancement is DNA barcoding, which is a powerful tool to identify a species with accuracy.

Genomic approaches in biodiversity research

Genomic approaches in biodiversity research: Genomic approaches routinely used in biodiversity research include DNA barcoding/meta barcoding, reduced representation DNA techniques, transcriptome sequencing (RNA-Seq), and whole-genome (re-) sequencing. Successful execution

of these approaches depends on the quantity and quality of the available biological material, laboratory and bioinformatic skills, feasibility and costs, and on the quality and completeness of the available reference genome database. We reviewed these common approaches, including their specific merits and limitations. Since the quality of the starting biological material is a major consideration in applying genomic approaches, we also review approaches that use non-invasive sampling.

DNA Barcoding and Metabarcoding: DNA barcoding has become a standard and efficient method for species identification and biodiversity monitoring. Initially, DNA barcoding was based on the mitochondrial cytochrome C oxidase and wildlife forensic investigations. Identification of species through DNA barcoding is precise as sequence information coming from sequencing can reveal the presence of a marker, which is representation of a particular species without any deviation. For instance, aquatic organisms such as fishes are identified by presence of cytochrome c oxidase (subunit COI) of mitochondrial DNA³⁰. This explains the existence of a particular species in an environment. Similarly, in the case of plants, coding regions present in the chloroplast that are part of genes, *rbcl* and *matK*, and, internal transcribed spacer (ITS) in rDNA for fungal species serve as barcodes respectively^{9, 27}. In the case of fishes, metabarcoding has become valuable tool for monitoring fish and amphibian species and this approach relies on amplifying genes using primer pairs and their subsequent analysis. The use of eDNA metabarcoding for species and genus level identification of aquatic plants, and also the utility of metatranscriptomics to study the relatedness and expression profiles were reviewed in the past.^{6, 21} In case of microbes, soil and water samples can be used for detecting presence of microbial species, for example, to identify methane producing microorganisms flourish in an environment rich of substrates that support methane production in anoxic environment. Metagenomic approaches are effective for this purpose. Furthermore, iBOL is another major initiative (Barcode of Life; <https://ibol.org/>) for studying biodiversity, species identification and interaction among insect species in the United Kingdom (UK). For example, Bioscan project of iBOL studies genetic diversity of 1,000,000 flying insects from across the UK using Barcoding and the DNA barcode data pertaining to these insect species. In addition to barcoding data, NGS technology in combination with bioinformatic methods can provide a clue about understanding genetic adaptation of an organism. However, the prerequisite for storing huge sequence data is a well-established bioinformatics platform and a collaborative research environment, so, the knowledge gained in this effort is shared with fellow counterparts. The genetic data obtained from various sources can be integrated for drawing inferences about species evolution and guide conservation efforts. Therefore, bioinformatics helps us to look at molecular level and understand more about management strategies of the species under conservation.

Reduced Representation DNA sequencing (RRS): Reduced representation DNA sequencing referred to as genotyping-by-sequencing (GBS) or restriction-site associated sequencing (RADseq) is another genomic strategy for assessing biodiversity (Figure 1). GBS approach provides sufficient genome-wide data in large sample sizes at low cost. The technique simply employs restriction endonucleases and size selection captured by hybridization using probes and provides adequate data to estimate genetic diversity, population size, population structure etc. GBS approach was successfully adopted to estimate within population diversity of pin-footed goose population.³² In another study, the applicability of GBS approach for unravelling evolutionary history of Torrey Pine (*Pinus Torreyana Parry*).²⁶ This study demonstrated substantial evidence for the application of genomic approaches for estimating genetic diversity and relatedness among individuals.

Whole Genome Sequencing (WGS): WGS briefly involves fragmenting the entire genome into fragments of varying size, sequencing these fragments by any NGS method, assembling the sequenced fragments back to its earlier state by various bioinformatic methods. The WGS approach also identifies genomic variants (Figure 1). It is a powerful approach to elucidate recombination, introgression, genomic regions under selection, admixture and to eventually study the evolutionary history of a species. Additionally, studies related to identifying underlying mutations that lead to tumorigenesis are also carried out by WGS approach²². A recent review, applications of WGS approach in studying genetic variation, population structure, biodiversity, genetic improvement, conservation efforts were elaborated²⁸.

RNA-Seq: RNA-Seq experiments focusses on quantifying gene expression based on transcript abundance and the typical workflow involves isolation of RNA, cDNA preparation, sequencing and analysis. RNA-Seq quantifies gene expression and relatively efficient when compared to microarray technology and qPCR-based methods which measure only single transcript. Various sequencing platforms are available for RNA sequencing such as PacBio single-molecule real-time (SMRT) sequencing which does not necessitate the PCR amplification and exploits sequence data to reveal functional variation (Figure 1) existing within and between species. This helps in identifying adaptive phenotypes and populations that are vulnerable. A few other studies related pangenome portrayed genes involved in showing resilience to stress and suggests their adaptive role in acquisition of ability to quickly respond to selective pressure¹³. A similar effort made by USDA-ARS to release Asian giant hornet complete genome and to make it available.

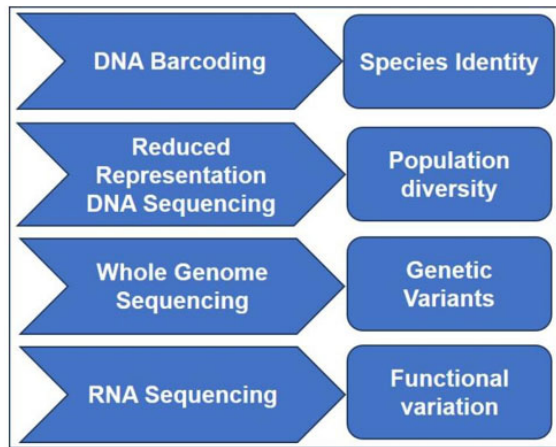


Figure 1: Showing application of genomic tools in species protection

Role of genomics in conservation: The use of genomic information in biodiversity conservation started in recent times, although it was not as rigorous as is the case of molecular breeding efforts in plants or animals. The integration of genomics with biodiversity conservation is promising as a greater number of plant and animal species are sequenced as part of genome projects. The genome projects provide information about genetic status of the population, number of genes and gene products, regulatory regions, mutations and other functional aspects of the genome. This information may lead to the understanding of genome organization and genome evolution which would otherwise be difficult by employing classical genetic approaches. Nevertheless, marker density is a limiting factor in genome projects restricting large number of genetic loci to be assayed due to limited resources. Another constraint is sequencing cost, genome size of individual and complexity in carrying out analysis of genetic datasets and lack of a reference genome. However, genomic approaches provide information about genetic parameters including genetic diversity of the population, population structure and breeding value of the species, as these parameters would explain reasons for threat to the community which may sometimes cause population to become extinct. A few other aspects that genomics can explain are, species delineation in the context of admixture, necessary adaptive alleles that a species precisely can possess for its existence in the changing environmental scenario. Another critical genetic parameter that influences a species existence per se, its fitness and patterns of inbreeding.

Application of genomic data for defining conservation units: As mentioned above, genomics can provide valuable information about various genetic parameters; however, it is important to define a conservation plan and/or a strategy and to clearly define conservation units such as species, subspecies and distinct populations as precisely as possible. Another challenging tasks for developing conservation strat-

egy is to identify a population or group as distinct based on traditional genetic distance methods and based on evidences about reproductive isolation. Traditional genetic techniques can define conservation units; however, this becomes complicated in an evolutionary system with a history of admixture. Admixture and introgression can complicate process of defining conservation units explicitly. Therefore, adoption of genomic approaches have proved to be effective in understanding invasion of neighbouring populations at molecular level. Therefore, one may need to identify key genomic approaches that can be integrated with conservation genetics.

Comparative genome mapping is also proved to be valuable and applied already to great extent in case of crop science. Comparative genome mapping provides information about species evolution. It would identify important traits of the population/species that are important for existence of the species, genetic identity and fitness. It also hints about traits that are distinct among closely related populations or species and population with difference in allele frequency. These traits are identified as conservation units which form a basis for the biodiversity conservation. For instance, in the case of animals such as giant panda and marbled murrelets, diversity at major histocompatibility complex (MHC) genes associated with adaptive immunity²⁴ was included as conservation management units. Comparative genomics can also aid in studying disease threat due to loss of diversity at specific loci and loci involved in disease vulnerability or increase disease threat.

Phylogenetic approaches are also being used to identify evolutionarily distinct species (e.g. evolutionarily distinct, globally endangered (EDGE) species. Introgression of genes by hybridization is another threat to biodiversity and species identity and this can also be quantified by comparative genomic approach such as to what extent, introgression occurred within and between threatened species. Molecular approaches may also identify genomic regions that are more diverse and variable, so this may allow us to include these regions in conservation management. Another significant effort in conservation efforts is identifying adaptive alleles. This is achieved by association mapping to aid conservation geneticists to set their priorities and make decisions when populations are protected from disappearing from the ecological niche.

Genomic data defines species identity and marker-trait associations: The main objective of adopting genomic approach in biodiversity conservation should be to identify molecular markers that can clearly distinguish between parent species and the proportion of ancestry in hybrid individuals. This precision can be enhanced only when we can increase marker density across the genome of an individual under study. However, as mentioned already, the genome size is another limiting factor in developing as many markers as

possible, in spite of fact that, NGS has helped us in overcoming these difficulties. It is also important to integrate genomic data with phenotypic data to derive meaningful and valuable ecological trait information as this helps in understanding environmental consequences of genetic introgression.

Association mapping: Association mapping (or) linkage disequilibrium (LD) mapping initially developed for human genetics can also be a valuable tool for preserving populations.^{3, 12, 18} Association mapping relies on decay of LD present in a population and is especially useful in unravelling association between phenotype and alleles of interest, in this context, genetic loci underlying resistance to disease has a necessary consequence in making the population resilient to diseases. In a recent study, the benefits of association mapping in identifying genetic variants have been discussed with respect to *Tasmanian devil* which is at risk of extinction due to devil facial tumor disease (DFTD).

Genome-wide association studies (GWAS) take genome-wide markers into consideration and identifies SNPs associated with complex diseases and traits. These genomic regions can be validated later by targeted genotyping of SNPs. Once the alleles of interest are identified, these markers can be deployed into breeding program for introgression into specific genotype. However, species barriers have to be taken into consideration when aimed to transfer into target species. In the case of conservation of marine diversity, coral regions can be targeted as the coral regions are under great threat due to pollutants and climate change. Global warming conditions may further prevent the marine species from inhabiting corals. In this direction, reported candidate loci in reef-building corals for heat stress response in association analysis and identified alleles associated with heat tolerance.¹ Scientists incorporate allelic frequencies in the models to predict coral's resistance changing environmental conditions including heat in the oceans. Similarly, in another study²³, genomic variants associated with drought damage were also identified by GWAS approach in European beech (*Fagus sylvatica*), which is a key stone species in temperate forests. The genetic diversity and level of inbreeding of species are important factors to prepare a conservation plan to determine, whether a genetic rescue program would help for the species survival or not. High inbreeding depression would lead to reduced genetic variation which causes severe phenotypic defects. Therefore, these challenges have to be considered when aimed at improving population in terms of fitness and population size.

Therefore, molecular approaches need to be utilized in population studies which may improve efforts for protecting their genetic identity. As seen in the case of genetic study of tiger (*Panthera tigris*) and leopard (*Panthera pardus*) in India, use of species-specific primers was adopted to study the population size for better conservation management practices for

these animals²⁵. Similarly, in the case of plants also, molecular markers such as SSRs, SNPs and Diversity Array Technology (DArT) markers can be routinely adopted in studies related to genetic diversity and conservation strategies. Genomic approaches are also useful in providing information on invasive species. For example, SNP data allowed to trace the origin of invasive species in black locust (*Robinia pseudoacacia*), a North American native species that invaded Europe in the 19th century.⁴

Role of CRISPR based methods: Ever since gene editing technologies such as Zinc-finger nucleases, TALENs, CRISPR-Cas9 and base editing were developed, introducing desirable changes in the specific genes has become a reality. The chestnut trees represent first example of genome editing as part of restoration strategies wherein, disease resistance genes were transferred from wheat to enhance the adaptability into the new environment.²¹ The application of CRISPR/Cas9 in case of plants was also reviewed in another report, however, the application of CRISPR/Cas9 for conservation biology is not clearly mentioned.¹⁷ CRISPR-based methods can also be used for detecting specific organisms in environmental DNA samples. In a recent study, CRISPR-Cas13a platform SHERLOCK (Specific High-sensitivity Enzymatic Reporter unlocking) enabled the accurate taxonomic identification of fish species.²

CONCLUSION

Despite the fact that, the dependence of ecosystem's health on species richness, rapid growth of human population, land reclamation, aquaculture and agricultural practices are the leading causes of species extinction and biodiversity loss around the world. So, the conservation of biodiversity has become paramount of importance for livelihood of people. NGS technology accelerated whole genome studies resulting in identification of millions of genetic variants like SNPs, Indels and CNVs which are useful to study population structure, genome diversity, genome organization for understanding genome evolution. In addition, integrating NGS with conservation biology would therefore result into rescue of species becoming extinct and will help conservations plans for preventing biodiversity loss. So, genomic technologies are valuable tools for conservation researchers, helping them to provide essential scientific information to managers and policy makers. Approaches have a long history of use in genetic biodiversity conservation, but the transition to genomic technologies is only just beginning.

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Table 1: Showing different sequencing technologies available

NGS technology	Sequencing principle	Read length
Solexa/454	Pyrosequencing	400-500 bp
ABI-Solid	Solid sequencing	2 × 50 bp
Illumina	sequence-by-synthesis	Up to 300 bp
Ion Torrent	Semiconductor sequencing	Upto 400 bp
PacBio	SMRT sequencing technology	10-15 kb
Oxford Nanopore technologies (ONT)	Single molecule sequencing	6-8 kb

Table 2: Showing bioinformatic tools available for sequence analysis

Assembler	Bioinformatic tool	Algorithm approach	Sequencing application
de novo assemblers	SOAPdenovo	<i>de Bruijn</i> graph	Genome assembly
	Velvet	<i>de Bruijn</i> graph	Genome assembly
	ALLPATHS-LG	<i>de Bruijn</i> graph	Genome assembly of large and small genomes
	ABYSS	<i>k-mer</i>	Genome assembly
	Bowtie	Burrows-Wheeler alignment	Genome assembly
Mapping based assemblers	AMOScmp	Homology guided	Genome assembly
	Oases	Read mapping	Transcriptome assembly
Genome alignment	MUMmer	Suffix tree data structure	Genome alignment (for identification of inversions)